5 Summary and future perspectives

The LI-cadherin (cadherin-17) is a structurally divergent member of the cadherin superfamily, with seven rather than five extracellular cadherin repeats, exclusively expressed in the small and large intestine but not in the upper gastric tract. Although lacking from the healthy gastric epithelia, LI-cadherin becomes strongly expressed during intestinal metaplasia and in gastric adenocarcinomas.

In this thesis, the first analysis of the mechanisms regulating murine and human LI-cadherin expression was performed. The transcriptional start site was identified by 5'-RACE and primer extension analysis. Activity of 2.8 kb of the 5'-flanking region was tested with GFP and luciferase reporter gene constructs in gastrointestinal cell lines.

A comparison of the human and murine 5'-flanking region revealed putative binding sites for the transcription factors AP-1, Cdx2, HFH3, HNF-1, GATA-1, 2, 3, OCT1, RFX1 and cMyb. The Cdx2 and HNF-1 binding sites were both conserved in mouse and human, while the GC-box was only found in the murine 5'-flanking region.

Deletion analysis was used to identify regions of the LI-cadherin promoter required for activity in transfected intestinal cells. The results indicated that promoter activity was dependent on positive regulatory elements located within only 55 nucleotides directly upstream of the transcriptional start site. EMSA and supershift assays identified the hepatocyte nuclear factor HNF-1 and Sp1 as interacting factors within this region. Further upstream, two binding sites for the intestine-specific homeodomain protein Cdx2 were detected. It was shown that only the more distal site interacted with Cdx2.

Mutational analysis demonstrated a positive regulatory role for HNF-1 and Cdx2. While the GC-box was lacking in the human sequence, function in mouse was indicated through mutational analysis of the murine GC-box in reporter gene assays:

Examination of fourteen cell lines derived from eight different tissues and three species showed that the promoter was highly active in some selected cell lines originating from intestine, liver, pancreas, stomach as well as lung, whereas other cells from these tissues were not able to activate the promoter.

In five of fourteen cell lines, the activity of the LI-cadherin promoter coincided with expression of endogenous LI-cadherin.

Two cell lines activating the promoter did not express LI-cadherin, while two other cell lines containing endogenous LI-cadherin were not capable of activating the promoter. Five cell lines did not express endogenous LI-cadherin, nor did they activate the promoter. Notably, the human cell lines Caco2 and HepG2 depicted a 1.6 to 1.8-fold higher reporter gene activity than the murine cell line STC1, indicating similar transcription factors being engaged in murine and human LI-cadherin activation.

The characterization of the LI-cadherin promoter *in vitro* provides material for further studies. Funds have already been granted to study the regulation of specific LI-cadherin expression *in vivo*. This project uses transgenic models to identify the regions of the murine LI-cadherin gene essential for tissue specificity and the expression of LI-cadherin during embryonic

development. In addition to the 2.8 kb 5'-flanking region, the complete intron 1 is going to be analyzed to see whether intron sequences are involved with the regulation, as they are with the transcriptional regulation of the E-cadherin gene (Hennig et al., 1996).

The role of Cdx2 for the expression of LI-cadherin in normal and tumorigenic processes in the gastrointestinal tract should be closely studied, since not only is the activity of the LI-cadherin promoter *in vitro* regulated by HNF-1 and Cdx2, but Cdx2 has also been shown to be up-regulated in intestinal metaplasia in the stomach (Bai et al., 2002).